The Effect of the Extirpation of the Thyroid upon the Thymus and the Pituitary Glands of Rana pipiens

by

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Date

Department of Zoology.

The Effect of the Extirpation of the Thyroid upon the Thymus and the Pituitary Glands of Rana Pipiens.

This problem was worked out in the laboratories of the Zoology Department of the University of Kansas under the direction of Dr. B. M. Allen in connection with other problems concerning the experimental removal of glands of internal secretion from Rana pipiens. Due credit is given for advice, material, and assistance; all of which have been a great help.

The thymus and the pituitary glands of a number of Rana pipiens in different stages of development were measured and drawn with camera lucida and comparisons made with thyroidless specimens.

The thymus is a paired gland found on the sides of the head of the frog just posterior to the tympanic membrane. In section the thymus appears as a round, compact mass of deeply staining cells with a darker outer portion and an inner part that stains somewhat lighter. The characteristic thymic corpuscles are found in the frog thymus. The general appearance is that of lymphoid tissue but is characterized by the thymic corpuscles. The thymus usually degenerates when sexual maturity is reached but may last through the life of the individual. The thymus is now considered as of lymphoid character but with the function of an organ of internal secretion that has to do with normal growth and sexual develop-

ment. The thymic corpuscles are interpreted as the portions that have the secretory function. (Hammar and Bell) It is regarded as the origional source of the leukocytes. (Beard 1900) This theory however is not generally accepted. There is much controversy as to the thymus being essential to life. Some writers say it is not essential to life but its removal has an effect upon the sex glands. Gudernatch(Amer. Jour. Anat.15,4,1914) says that thymus feeding increased the growth, but retarded differentiation in frog tadpoles.

The material was furnished by Prof. B. M. Allen and was used by him in his experiments upon the removal of the thyroid.

The controls consisted of a number of tadpoles raised under the same conditions as the thyroidless and a number of normal frogs in various stages of metamorphosis and in the mature frog stage.

In the stages up to and including the 24mm. stage the specimens were prepared for study by sectioning the head portion which contains the pituitary and the thymus. The sections were stained with Haidenhain's iron alum-haematoxylin and eosine.

The glands were measured by taking the two dimensions from each section in the series and by counting the sections and multiplying by the thickness to get the other dimension. The average of each dimension times the average of the other dimensions furnishes a means of comparing the glands for size. With an eye piece micrometer the longest diameter of the gland in that particular section was measured. Then the micrometer was

turned at right angles to the first measurement and the reading recorded.

In the later stages the desired measurements were obtained in a different manner. The specimens were too large to be readily sectioned through the head. Accordingly the specimen was pinned on paraffin under a binocular miscroscope. The thymus was then exposed and dissected. Each gland was then put in a shallow dish and drawn with a camera lucida. The gland was first drawn showing the outline in a plane perpendicular to the shorter diameter. Then it was set up edgewise in a small groove cut in the paraffin so that the second drawing showed the outline in the plane of the shorter diameter. After each drawing a scale was placed under the microscope and the scale of magnification for that particular drawing indicated on card with the drawing. This furnished a check on the adjustment of the camera lucida.

The pituitary gland was taken from the same specimens as was the thymus. Some of the specimens were injured in manipulation and in those cases the measurements of the destroyed glands can not be given. The procedure in the measuring of the pituitary was the same as for the thymus until it was advisable to measure the dissected gland. In these stages the pituitary is much smaller than the thymus and harder to handle. The most satisfactory method was to dissect the whole brain and take it out of the specimen with the pituitary attached. The dura mater was carefully removed to expose the gland as much as possible. The gland was first drawn from

its ventral aspect and then from the lateral aspect by the same instruments as were used in measuring the thymus.

The same methods were applied to the thyroidless specimens which had been prepared by Dr. Allen in the spring of 1916. These specimens were killed as needed over a period extending from a few days after removal in April 1916 to March 1917. The oldest experimental animal was killed early in March. None of the thyroidless tadpoles metamorphosed into frogs and the comparisons in the later stages are between thyroidless tadpoles and normal frogs of about the same age as the experimental animals.

In determining the difference in size of the glands in the thyroidless and the control animals it is necessary to use a standard of comparison rather than the actual volumes because of the method adopted. The three dimensions obtained by the method outlined in the preceding paragraphs were multiplied together which gives the volume of a parallelipiped of those dimensions. Since conditions were uniform in the measuring the same relative error would apply to all. When the glands vary much from the average of the class to which they belong due to irregular contour the difficulty can be adjusted by reference to the drawings. **Correction for body length was made by dividing the volume by the body length.**

Results. Migration of the thymus glands.

In the course of this work it was found that the thymus glands of normal animals migrate in the process of metamorphosis. In normal tadpoles the thymus gland is found ventral

Measurements of Controls

Specimen	Total length	Body length	Pituitary Anterior lobe sagittal	Pituitary transverse	Pituitary perpendicular in sagittal plane	Right thymus sagittal	Right thymus transverse	Right thymus perpendicular in sag. plane	Left thymus sagittal	Left thymus transverse	Left thymus perpendicular in sag. plane
1	10.5		.110	.133	.041	.230	.094	.134	.220	.094	.134
2	14		.200	.163	.045	.360	.111	.165	.360	.111	.165
3	24		.280	.238	.075	.850	.310	.434	.870	.310	.434
4			.180	.273	.071	.330	.254	.459	.340	.317	.542
5	62	27	.375	.392	.107	1.967	.535	.950	1.967	.670	.678
6	72	27	.428	.535	.160	2.178	.893	1.071	2.017	.750	1.107
7	73	30	.500	.642	.178	2.464	.857	1.250	2.392	.857	1.250
8	82	31	.410	.678	.142	2.500	.625	1.142	2.071	.892	1.107
9	64	32	.571	.714	.142	2.464	.714	1.142	2.500	.642	1.232
10	74	34	.500	.714	.214	2.250	.892	1.250	2.250	.892	1.250
11		23				1.089	.571	.714	1.089	.660	.785
12		24				1.303	.428	.857	1.107	.500	.857
13		24	.428	.642	.178	1.428	.714	.821	1.392	.642	1.071
14		24	.357	.357	.142	1.125	.464	.750	1.125	.500	.723
15		24				1.107	.571	.785	1.107	.571	.857
16		24	.428	.535	.142	1.535	.821	1.107	1.964	.821	1.071
17		46	.607	1.125	.232						
18		46				1.857	.250	.714	1.948	.232	.767
19		48				1.535	.178	.857			
20		50	.821	1.178	.250	2.438	.393	.642	2.328	.393	.892
21		45	.714	1.000	.214				1.607	.428	.714
22		48	.785	1.303	.196						
23		50	.785	1.250	.214	1.964	.214	.928	1.821	.250	.857
24		51	.875	1.142	.250	2.357	.285	.857	2.392	.285	.857

Measurements of Thyroidless

1	10.5		.060	.154	.041	.170	.104	.138	.160	.104	.138
2	13		.090	.188	.065	.180	.178	.216	.180	.178	.216
3	24		.280	.192	.074	.760	.236	.255	.800	.255	.360
4	85	36	.569	1.020	.300	1.458	.341	.900			
5	45	16.2	.482	.482	.110						
6	97		1.125	1.000	.410				3.196	.428	1.071
7		33	.892	1.017	.457	1.892	.446	.875	2.857	.446	.946
8	123	43	1.017	1.232	.321				3.250	.535	1.446
9	76	34	.607	.821	.357	2.678	.482	1.178	3.142	.446	1.196
10	102	40	.875	1.375	.303	2.750	.410	.842	2.785	.410	.835

Volumes of Controls

Specimen	Total length	Body length	Anterior lobe of Pituitary	Right thymus	Left thymus
1	10.5		5	2	2
2	14		1	6	6
3	24		4	114	117
4			3	38	58
5	62	27	15	999	809
6	72	27	36	2083	1674
7	73	30	57	2639	2562
8	82	31	39	1784	2044
9	64	32	57	2011	1977
10	74	34	76	2508	2508
11		23		443	563
12		24		530	474
13		24	48	837	957
14		24	18	391	406
15		24		496	496
16		24	32	1395	1730
17		46	158		
18		46		331	344
19		48		234	
20		50	241	615	816
21		45	154		
22		48	200		
23		50	209	390	390
24		51	249	575	584

Relative Volumes of Thyroidless

1	10.5		3	2	2
2	13		1	6	6
3	24		3	45	73
4	85	36	174	447	
5	45	16.2	41		
6	97		461		1465
7		33	313	693	1205
8	123	43	402		2621
9	76	34	177	1520	
10	102	40	364	949	953

Relative volumes in proportion to body length.

Thyroidless Controls

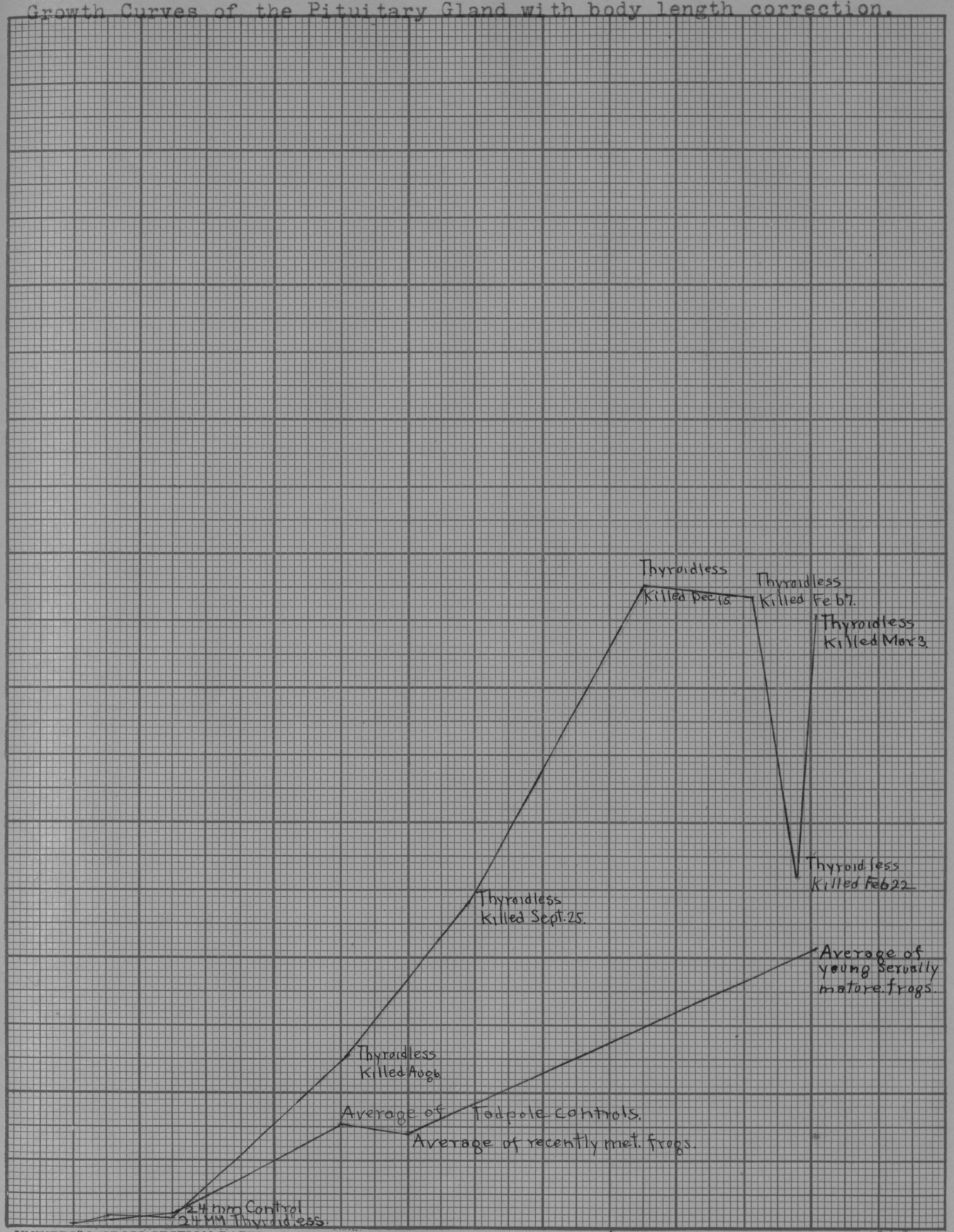
Anterior lobe of the Pituitary gland.

Specimen	Total length	Body length	Volume	Volume:: body length
1	10.5		.3	3
2	13		1.9	15
3	24		3.9	16
4	85	36	174	483
5	45	16.2	41	254
7		33	313	950
8	123	43	402	935
9	76	34	177	520
10	102	40	364	911

Specimen	Total length	Body length	volume	volume:: body length
1	10.5		.5	5
2	14		1.4	10
3	24		4.9	20
5	62	27	15	58
6	72	27	36	135
7	73	30	57	190
8	82	31	39	127
9	64	32	57	180
10	74	34	76	224
13		24	48	203
14		24	18	75
16		24	32	135
17		46	158	344
20		50	241	483
21		45	154	344
22		48	200	417
23		50	209	419
24		51	249	489

Average pituitary of normal tadpoles	Specimens 5 to 10	152
Average pituitary of young frogs	Specimens 13 to 16	138
Average pituitary of adult frogs	Specimens 17 to 24	416

Growth Curves of the Pituitary Gland with body length correction.

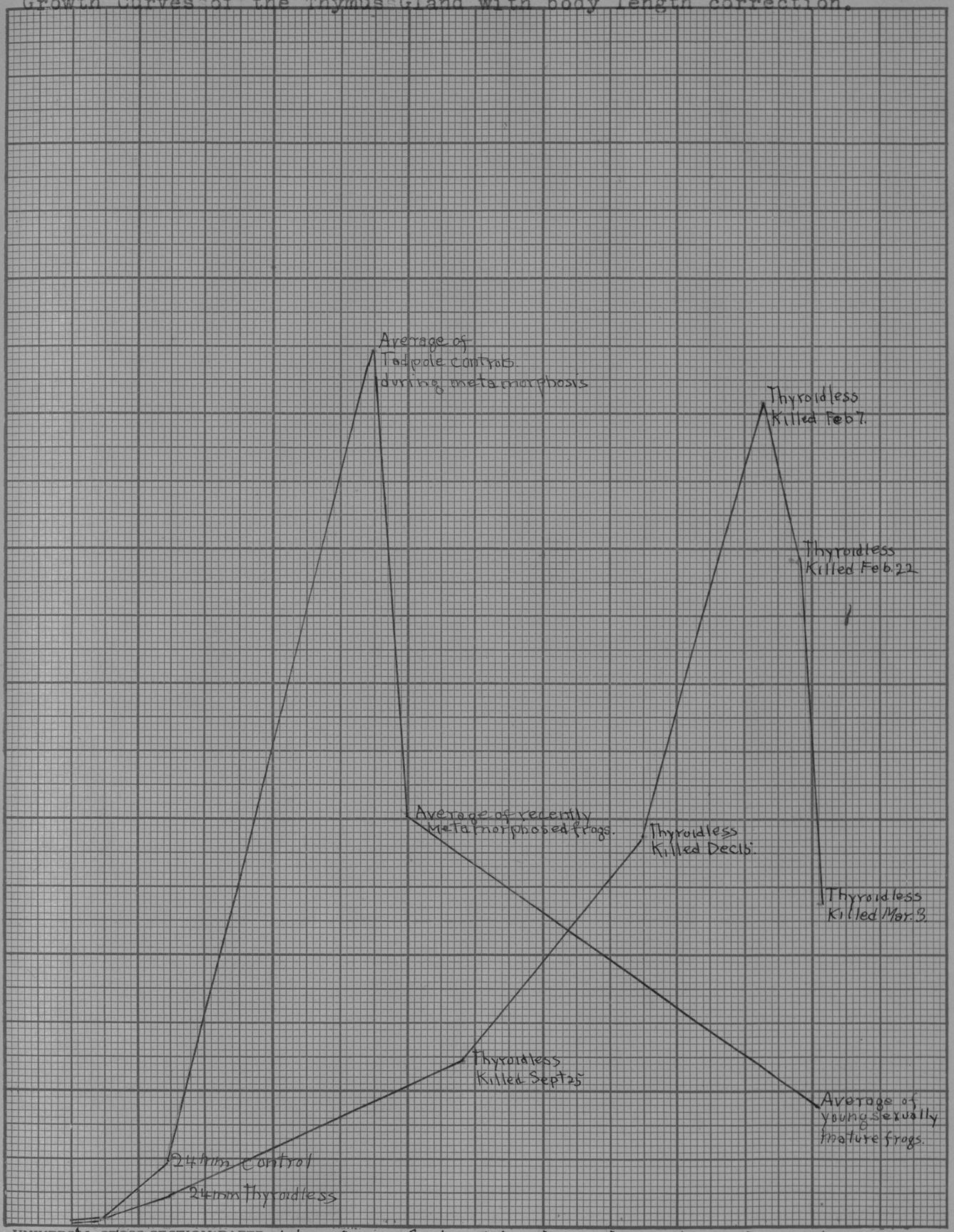


Relative volumes in proportion to body length.

Thyroidless			Thymus gland				Controls		
Specimen	Total length	Body length	Relative volume	Relative volume:: body length	Specimen	Total length	Body length	Relative volume	Relative volume:: body length
1	10.5		47	4	1	10.5		56	5
2	13		138	10	2	14		131	9
3	24		1191	49	3	24		2314	96
4	85	36	8949	248	5	62	27	18091	670
7		33	18990	575	6	72	27	37577	1391
8	123	43	52424	1219	7	73	30	52019	1733
9	76	34	31966	940	8	82	31	38293	1235
10	102	40	19027	475	9	64	32	39884	1234
					10	74	34	50175	1475
					11		23	10755	438
					12		24	10044	418
					13		24	17941	747
					14		24	7976	332
					15		24	9923	413
					16		24	31254	1302
					18		46	6762	147
					19		48	5285	110
					20		50	14312	286
					21		45	9825	218
					23		50	7801	156
					24		51	11596	227

Average thymus of normal tadpole	Specimens 5-10	1293
Average thymus of young frogs	Specimens 11-16	608
Average thymus of adult frog	Specimens 18-24	182

Growth Curves of the Thymus Gland with body length correction.



and posterior to the eye and attached to the skull by connective tissue. In the metamorphosing frog as the head is taking on adult characters the thymus gland is found to have migrated posteriorly to a point just ventral to the tympanum. In the adult frog it is found in a mass of connective tissue under the depressor mandibuli muscle, thus coming to lie ventral and posterior to the tympanum. The thyroidless tadpoles still have the glands close to the eye and anterior to the tympanic membrane.

When the frog reaches the stage of sexual maturity, the thymus is flattened. This is due to its position between the muscles. In going from the tadpole to the recently metamorphosed frog stage it will be noticed in the drawings that there is sudden decrease in the size of the thymus glands and at the same time the glands become more globular. This is attributed to the loss of water from the tissues at metamorphosis.

The pituitary and the thymus glands vary much in size and these differences can not always be explained by a corresponding difference in body length. This is strikingly shown by examination of the class of normal tadpoles Specimens No. 5-10, which were killed in July. Control No. 5 has a body length of 27 mm. and a pituitary gland represented by 58 as determined by the method explained above. Control NO. 10 has a body length of 34 mm. and a pituitary gland represented by 224. This shows that the larger tadpole has the larger

pituitary. Contrary to this, control No. 7, a 30 mm. tadpole has a pituitary gland represented by 190 while control No.8, a 31 mm. tadpole has a pituitary gland represented by 127. In this case the smaller tadpole has the larger pituitary gland thus showing individual variation. In these cases the thymus glands are larger in the animal containing the larger pituitary gland.

This must be attributed to individual variation which may be due to a number of causes. The effect of individual variation is overcome by taking the average of the class. The thyroidless specimen of given age is compared with the average of the controls of that age instead of one individual control.

In some specimens one thymus gland is larger than the other of the pair. A case of this is found in Control No. 6 For the most part the pair of thymus glands approximate each other in size. Thyroidless tadpoles No. 7 shows a marked difference in the size of the two thymus glands. This variation however is found in both thyoidless and control specimens.

Comparisons of the pituitary and the thymus glands in young tadpoles.

In comparison there is nothing particularly striking in the 10.5, 13, and 24 mm. total length, respectively except in the growths of the normal thymus. Both the pituitary gland and the thymus are slightly larger in the 10.5 mm. control tadpole. The 13 mm. throidless tadpole has somewhat

larger glands than the 14 mm. control tadpole. The 24 mm. control tadpole has better developed glands than the 24mm. thyroidless tadpole. Here it is noticed that the normal thymus has made a markedly greater increase than that of the thyroidless tadpole. The thymus gland of the thyroidless is represented by 49 and that of the normal tadpole by 96. This proves nothing at this stage but it gives a hint as to the further growth of the thymus gland in the larval frog.

Comparisons of the pituitary and the thymus glands in metamorphosing normal tadpoles and thyroidless tadpoles of about the same age.

The period of normal metamorphosis for Rana pipiens extends from June to August, depending on the time at which the eggs were laid, the season, the food, etc. The thyroidless tadpole No.4 was killed Sept. 25, 1916 and had a body length of 36mm. This specimen was killed soon after the time when normal metamorphosis took place. The largest of the metamorphosis control tadpoles No. 10 which had a body length of 34 mm. The average body length of these controls was 30.16 mm. The thyroidless tadpole killed Sept. 25, had a pituitary gland which with body length correction was represented by 483. The average of the pituitary glands of the normal metamorphosing tadpoles on the same basis was 152. The pituitary gland of the thyroidless tadpole was much larger than the average for the metamorphosing normal tadpoles. This shows that the pituitary gland of the thyroidless specimen has made a marked increase over the normal pituitary gland. The

portion of the pituitary glands in question is the anterior lobe. Thyroidless tadpole No. 5, with a body length of 16.2 mm. was abnormally small. The pituitary gland was smaller than that of the Sept. 25. thyroidless specimen. When the pituitary gland of this specimen is considered with body length correction as were the glands of the control and the thyroidless tadpoles it has a relative size of 254. Even in this small thyroidless tadpole the pituitary gland is proportionally larger than in the control tadpoles.

The thymus gland of the tadpole killed Sept. 25 had a relative volume with body length correction of 248. The thymus glands of the control tadpoles were much larger and the average for the class was 1293. This shows that during the period of metamorphosis that the normal thymus gland is increased very much. The thymus gland of the control tadpole is therefore much larger than that of the thyroidless tadpole which is slightly older.

The striking thing shown in this stage is that the pituitary gland of the thyroidless tadpole is much larger than that of the control tadpoles and the thymus glands of the latter are a great deal larger than those of the thyroidless tadpole. These differences are clearly shown on the graphs.

The pituitary gland of the thyroidless tadpole killed Sept. 25 was represented by 483. The average for recently metamorphosed frogs slightly older than the metamorphosing tadpoles was 138. This shows that the pituitary gland is

smaller in the recently metamorphosed frog than in the thyroidless tadpole of the same age. The pituitary glands of the recently metamorphosed frogs are smaller than those of the normal tadpole.

The thymus gland of the thyroidless tadpole killed Sept. 25 was represented by 248. The average for the class of recently metamorphosed frogs was 608. This shows the thymus glands of the normal recently metamorphosed frog to be larger than those of the thyroidless tadpole killed Sept. 25 but much smaller than those of the normal tadpoles.

The thyroidless tadpole has a larger pituitary gland than the recently metamorphosed frog control but the metamorphosed frog has larger thymus glands. The difference is not so great as in the case of the normal tadpoles.

Comparisons of the pituitary and the thymus glands in young sexually mature frogs and thyroidless killed between Dec. 12 1916 and March 3 1917.

The thyroidless tadpole No.7 killed Dec. 15 1916 had a body length of 33mm. The pituitary gland was represented by 950 and the thymus gland by 575.

The thyroidless tadpole No.8 killed Feb. 7 1917 had a body length of 43 and was the largest of the thyroidless specimens. It had a body length of 43mm. The pituitary gland was represented by 935 and the thymus gland by 1219 on the relative standard. The thyroidless tadpole No. 9 killed Feb. 22 1917 had a body length of 34. The pituitary gland was

represented by 520 and the thymus gland by 940.

The thyroidless tadpole No. 10 killed March 3 1917 had a body length of 40 mm. The pituitary gland was represented by 911 and the thymus gland by 475. The pituitary gland of this specimen was larger than that of the Feb. 22 specimen but smaller than than of the Feb. 7 thyroidless tadpole. The thymus glands of this specimen were smaller than those of the other two thyroidless specimens killed a little sooner. There are not sufficient grounds however to say that the thymus glands are degenerating. That remains to be determined in some thyroidless tadpoles that have not yet been killed.

The controls for these older throidless tadpoles were a number of young sexually mature frogs that metamorphosed the preceding summer and were killed. Feb. 18-March 4. The average volume of the pituitary gland for this class of controls was 416. The pituitary gland in each of the four oldest thyroidless specimens was largerthan in the controls.

The pituitary glands of the four oldest thyroidless tadpoles are represented by 950, 935, 520 and 911 respectively. It is apparent here that the pituitary gland in the thyroidless tadpoles grew larger than did the pitiutary in a younger sexually mature frogs. This was especially noticeable when corrections were made for relative body length. It is remarkable for the thyroidless specimens were still in the tadpole stage while the frogs had metamorphosed the preceding summer.

The average of the thymus glands for the young frog controls was 182. This volume varied in the different specimens

from 110 to 286. The thymus glands of each of the three oldest thyroidless tadpoles is represented by 575, 1219, 940, and 475 respectively. It is clear that in none of the young sexually mature frogs were the thymus glands as large as in the four oldest thyroidless tadpoles. The thymus gland of the young sexually mature control frog is much smaller than that of the recently metamorphosed frog. The thymus degenerates as maturity is reached in most forms and this is what is happenig here. In the case of the thyroidless tadpoles this degeneration has not taken place and instead the thymus gland has developed more and has grown during a longer period of time.

It is interesting to note here that the thyroidless tadpole killed Feb.7 has well developed thymus glands in spite of the fact that the tadpole is sexually mature with many live sperm. The thymus gland has persisted after sexual maturity was reached.

The Graphical Representation of Volumes.

The number of thyroidless tadpoles used as a basis for the graphs was so small that the curves should be accepted as showing general features and not as showing the accurate details of growth. The volumes of the pituitary glands in both the thyroidless and the normal tadpoles do not vary much until the period of metamorphosis. At that time the line of the pituitary gland of the thyroidless goes up sharply. The line for the controls goes down slightly. The line for the thyroidless tadpoles stays up until the Feb. 22 specimen is

reached when it comes down only to go up again for the March 3 thyroidless tadpole. The line for the control specimens comes up gradually until the young sexually mature frog stage is reached. Th The great deviation in the volume of the pituitary gland of the thyroidless tadpole killed Feb.22 is probably due to individual variation and has no great significance.

The line of growth of the thymus glands in the thyroidless specimens goes up gradually until the Sept.25 specimen and then it mounts rapidly. It reaches its greatest height in the Feb.7 specimen and then drops. The sudden decline might mean degeneration but on account of the few thyroidless tadpoles observed it could not be determined.

The line for the normal controls goes above the line for the thyroidless at the 24mm. stage and mounts very hightduring the period of metamorphosis. It then comes down sharply to the recently metamorphosed frog stage and slopes off gradually to the young sexually mature frog stage.

Summary

1. The pituitary gland develops when the thyroid gland is extirpated and the anterior lobe reaches a larger size in proportion to body length than it does in a normal specimen. This is true in all stages up to sexually mature young frogs. In most cases the anterior lobe of the pituitary gland of the thyroidless tadpole is larger than that of the corresponding control even without reference to body length.

2. The thymus gland develops when the thyroid gland is extirpated. The thymus glands of normal controls are larger during the period of metamorphosis and immediately fallowing than at any other period of the frog's life.

3. The young sexually mature frogs have smaller thymus glands than thyroidless tadpoles of the same age.

4. The thymus gland is the largest in proportion to body length in normal tadpoles at metamorphosis.

5. The pituitary gland is the largest in proportion to body length in thyroidless tadpoles that are of the same age as the young sexually mature frogs.

6. The thymus glands of thyroidless tadpoles do not migrate to the position in which the thymus gland is found in adult frogs.

7. The thymus gland of the thyroidless tadpole does not degenerate like that of a frog that develops normally.

Explanation of Plate.

Magnification 28X. Reduced to 3/7.

Pituitary Glands of Thyroidless Tadpoles.

1 Pituitary gland of thyroidless tadpole No.5
2 " " " " " No.6
3 " " " " " No.7
4 " " " " " No.8
5 " " " " " No.9
6 " " " " " No.10

Pituitary Glands of N ormal Tadpoles

7 Pituitary gland of tadpole Control No.5
8 " " " " " No.6
9 " " " " " No.7
10 " " " " " No.8
11 " " " " " No.9
12 " " " " " No.10

Pituitary Glands of Recently Metamorphosed Frogs.

13 Pituitary of Control Frog No.13
14 " " " " No.14
15 " " " " No.16

Pituitary Glands of Sexually Mature Young Frogs.

16 Pituitary Gland of Control Frog No.17
17 " " " " " No.20
18 " " " " " No.21
19 " " " " " No.22
20 " " " " " No.23
21 " " " " " No.24

Thymus Glands of Thyroidless Tadpoles.

22 Left thymus gland Thyroidless No.6
23 " " " 24 Right thymus gland " No.7
25 Right thymus gland " No.8
26 Left thymus gland 27 Right thymus gland " No.9
28 " " " 29 " " " " No.10

Thymus Glands of Normal Tadpoles.

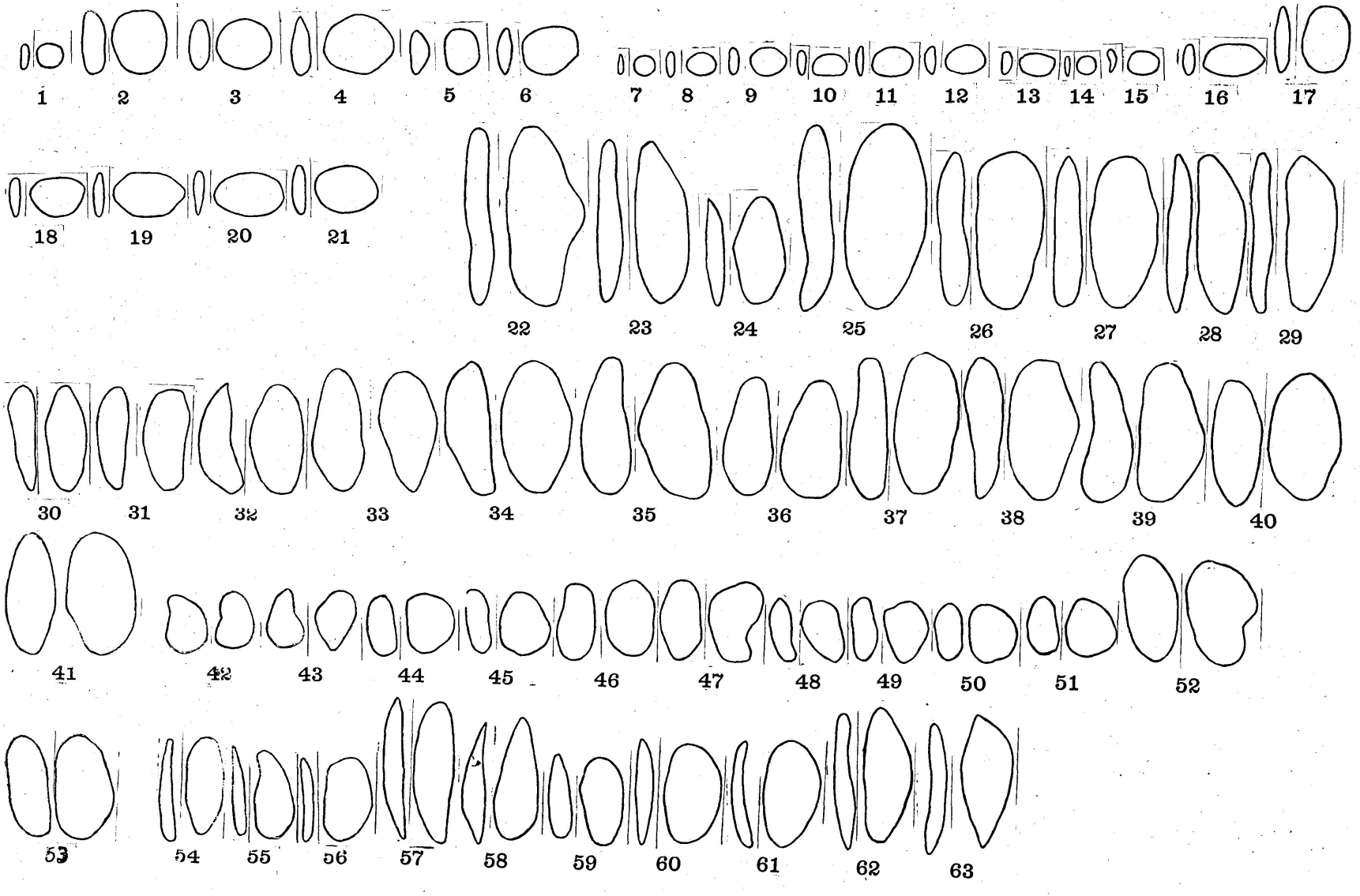
30	Left	thymus	gland	31	Right	thymus gland	Control No.5
32	"	"	"	33	"	" "	" No.6
34	"	"	"	35	"	" "	" No.7
36	"	"	"	37	"	" "	" No.8
38	"	"	"	39	"	" "	" No.9
40	"	"	"	41	"	" "	" No.10

Thymus Glands of Recently Metamorphosed Frogs.

42	Left	thymus	gland	43	Right	thymus gland	Control No.11
44	"	"	"	45	"	" "	" No.12
46	"	"	"	47	"	" "	" No.13
48	"	"	"	49	"	" "	" No.14
50	"	"	"	51	"	" "	" No.15
52	"	"	"	53	"	" "	" No.16

Thymus Glands of Sexually Mature Young Frogs.

54	Left	thymus	gland	55	Right	thymus gland	Control No.18
				56	"	" "	" No.19
57	Left	thymus	gland	58	"	" "	" No.20
59	"	"	"				" No.21
60	"	"	"	61	Right	thymus gland	" No.23
62	"	"	"	63	"	" "	" No.24


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
18 19 20 21
22 23 24 25 26 27 28 29
30 31 32 33 34 35 36 37 38 39 40
41 42 43 44 45 46 47 48 49 50 51 52
53 54 55 56 57 58 59 60 61 62 63